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RESEARCH ARTICLE

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A RAPID PROTOCOL FOR ISOLATION OF DNA FROM PADDY SEED AND HYBRID PURITY CONFIRMATION

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A B S T R A C T

A total of four microsatellite markers were used across two hybrids and 12 rice varieties for theircharacterization and discrimination. SeedDNA isolation protocol has been standardised and the same protocol was for the characterization of thirteen varieties and two hybrids. The popular rice hybrids viz., KRH-2 and KRH- 4 and used a rice microsatellite marker RM206, RM 234, which exhibits genotype specific amplification with respect to KRH2, to check for impurities. The PCR was successful with respect to the DNA isolated from single seeds and also RM 276 and by using this method we can detect the contaminants.

INTRODUCTION

Rice is the staple food for a large segment of the Asian population. It has been estimated that rice production in India as well as several other Asian countries must double by the year 2025 to meet the requirements of the increasing population. A self-pollinated crop like rice, one of the challenges is the production and supply of adequate quantities of pure seeds to the farmers. And maintenance of high level genetic purity of hybrid seeds and it is to exploit the moderate level of heterosis in this crop. It is estimated that for every 1per cent impurity in the hybrid seed, the yield reduction is 100 kg per hectare. Thus, there is a need for an assay to assess genetic purity of seeds that is both accurate and faster, so seed produced in the dry season can be released for commercial cultivation in the ensuing wet season (Ranjitha *et al*., 2016). The genuineness of the variety is one of the most important characteristics of good quality seed. Genetic purity test is done to verify any deviation from genuineness of the variety during its multiplications. Genetic purity test is compulsory for seed certification of all foundation and certified hybrid seeds.

Higher genetic purity is an essential prerequisite for the commercialization of any hybrid seeds. Besides, success of any hybrid technology depends on the availability of quality seed supplied in time at reasonable cost. Molecular marker applications like large-scale genotyping and hybrid rice (Yashitola*et al*. 2002) and other rice genotypes (Bligh 2000) purity assessments demands rapid isolation of high quality genomic DNA in a cost effective manner from a large number samples.

Several protocols (Chunwongse et al. 1993, Kang et al. 1998, Pal et al. 2001, Sharma et al. 2002) and commercial kits (Nucleon phytopure DNA isolation kit, Amersham Biosciences, USA) are available for DNA isolation from rice seed and grain, but they are expensive and/or cumbersome involving the use of relatively costly reagents like Chelex-100, proteinase K and are unsuitable for handling large numbers of samples. An ideal DNA isolation method should require only a small amount of tissue, involve simple procedures, use a minimal number and amounts of chemicals, should be rapid and yield reasonably good quality as well as quantity of DNA. We have standardized a modified Cetyltrimethyl ammonium bromide (CTAB) based procedure for isolation of high quality and quantity of DNA from single rice grain, seed and leaf tissue for deployment in hybrid seed and Basmati grain purity assessments and also for rapid genotyping in marker-assisted breeding programmes.

MATERIALS AND METHODS

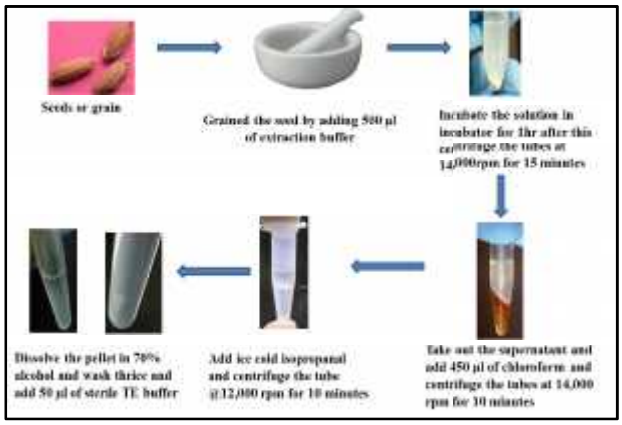
For molecular studies, genomic DNA was isolated from single seed taken genotype of the rice following CTAB (Cetyl Trim ethyl Ammonium Bromide) method. We isolated DNA from thirteen varieties and two hybrids. A sample set consisting of 400 single seeds of the popular rice hybrid KRH-2 and KRH- 4 (Collected from VC Farm Mandya) and used a rice microsatellite marker RM206, RM 234

These genotypes were subjected to classify for genetic diversity of rice with the help of different primers. Agarose gel electrophoresis was used to quantify DNA on the basis of molecular weight. The purified DNA was amplified in PCR with different SSR primers. The genotypes were subjected to screen for diversity.

Procedure

1. Dehusked the single rice seed and soaking the dehusked seed or grain in 500 µl extraction buffer (100 µl Tris-cl, P^H -8.2, 20mm EDTA pH 8.2, 1.20M NaCl, 2.2% CTAB and 3% PVP) for 45 minutes to 1 hr at 37-40^0 C in a sterile 1.5-2.0 ml microfuge tube and graind the sample by using sterile pestle till the tissue is disintegrated.
2. Then 450 µl of chloroform is added the contents are mixed gently for 5 minutes and centrifuged at 14000 rpm for 15 minutes at room temperature
3. Then the supernatant is transferred to a fresh sterile 1.5-2.0 ml microcentrifuge tube and the DNA is precipitated using an equal volume of ice cold isopropanol
4. The DNA is pelleted by centrifugation at 12000 rpm for 10 minutes at room temperature after centrifugation the supernatant is discarded
5. The DNA pellet is washed twice or thrice with 70% ethanol. The pellet is air dried for 2 hr.
6. Then dissolve the pellet in 50 µl of sterile TE buffer (10mM TrisHcl pH 8.2, 1mM EDTA pH 8.2).

NOTE: grinding the seed / grain without incubation in the buffer results in distinct DNA degradation while grinding the seed / grain after 45 minutes – 1hr incubation always gave good quality and quantity DNA.



RESULTS AND DISCUSSION

Isolation of seed DNA was Characterized and identification of cultivars are crucial to varietal improvement, release and in seed production programme. It is mandatory to maintain the genetic purity of hybrid seed for the successful crop production. Unambiguous characteristic pattern of hybrids can be obtained using DNA markers and had been termed as DNA fingerprinting. The use of DNA markers to obtain genotype specific profiles had distinct advantages over morphological and biochemical methods.

DNA concentration

The DNA from thirteen varieties and two hybrids were assessed for DNA concentration and it varied among the samples. The concentration ranged from MandyaSona (410 OD value) to KMP 128 (1420 OD value)(Table 1) and purity of the DNA varies from (1.797) to MTU 1001 (2.245). The biochemical markers such as isozyme and protein patterns are least influenced by the environment but exhibit limited polymorphism and often do not allow discrimination between closely related inbred lines (Lucchese et al., 1999; Amruta*et al*., 2016). DNA markers overcome most of these disadvantages of morphological and biochemical markers that can be useful to distinguish varieties and off types.

Table 1 DNA and its qualityobtained from different genotypes

Genotype	OD value @ 260/280	Purity
BI-33	682	2.068
MAS 946-1	513	1.783
TellaHamsa	442	2.133
Raksha	438	2.159
MTU 1001	573	2.245
MAS 26	440	2.200
CTH 1	575	1.797
KMP 201	633	2.144
MandyaSona 2	410	2.103
MTU 1010	455	2.364
CTH 3	695	1.972
KMP 128	1420	2.073
KRH4	998	2.249
KRH2	881	2.196
Rasi	915	2.116

The modified procedure used for the isolation of DNA from thirteen varieties and two hybrids.A sample set consisting of 400 single seeds of the popular rice hybrid KRH-2 and KRH-4 (Collected from VC Farm Mandya) and used a rice microsatellite marker RM206, RM 234, which exhibits genotype specific amplification with respect to KRH2, to check for impurities. The PCR was successful with respect to the DNA isolated from single seeds and also RM 276 and by using this method we can detect the contaminants were reliably (fig 2a and fig 2b).

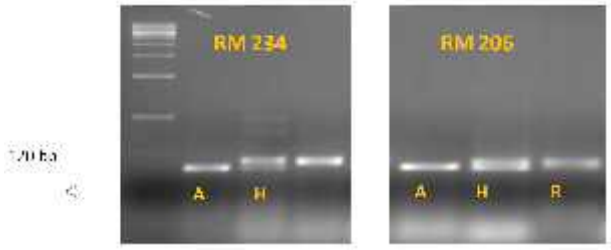


Fig. 2a Polymorphic profiles of SSR markers confirming hybridity of KRH-2 with (a) RM-234 and (b) RM-206

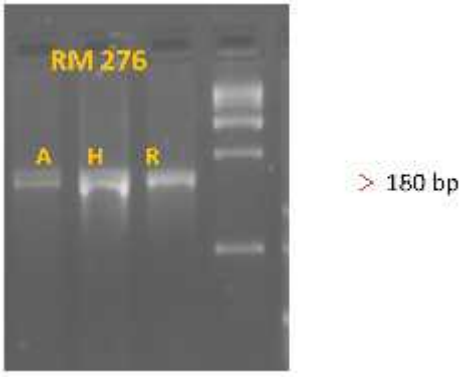


Fig. 2b Polymorphic profiles of SSR markers confirming hybridity of KRH-4 with RM-276

One of the critical aspects of our DNA isolation protocol is the incubation of seed/grain before isolation of DNA. We observed that grinding of seed/grain without incubation in the buffer results in distinct DNA degradation while grinding the seed/grain after 30-45 min incubation always gave good quality and quantity DNA. Incubation of seeds in the buffer softens the hard tissue due to imbibition, which helps in smooth and easy grinding. In the present study, we observed that our DNA isolation procedure is highly amenable for DNA isolation from half seed also. Hence, the DNA can be used for non-destructive analysis of segregating progeny since the selected remnant half seeds containing the embryo part can be germinated later. From each dehusked seed/grain of rice, we obtained 1.8-2.0 µg of DNA. The same protocol has also been tested and found to effective for isolation of DNA from leaf with slight modification. Fresh leaf tissue can be ground directly in extraction buffer using a spot test plate as per the procedure of Zheng et al. (1995). From 3 cm leaf piece, 4-5 µg of DNA can be isolated. In a modest laboratory, a team of 2-3 personnel could handle DNA extraction from about 800-1000 seed/grain of rice per day.

The isolated DNA was highly intact, devoid of shearing and comparable to those isolated using the protocols of Pal et al. 2001, Chunwongse et al. 1993 and Nucleon phytopure kit. This new and rapid protocol of DNA isolation from single seed/grain and leaf tissues is fast, consistent and inexpensive. Besides, this method does not involve the use of phenol, which is hazardous. We recommend this method for use in marker-based seed/grain purity assays and also for rapid genotyping in marker-assisted breeding programmes.

References

Amruta, N., Prasanna Kumar, M.K.,Narayanaswamy, S., MalaliGowda, Channakeshava, B.C.,Vishwanath,K., Puneeth, M. E. and Ranjitha,H. P. (2016).Isolation and Identification of Rice Blast Disease - Suppressing Antagonistic Bacterial Strains from the Rhizosphere of Rice. *Journal of Pure and Applied Microbiology*, 10 (2):1043-1054.

Bligh H. F. J. (2000). Detection of adulteration of Basmati rice with non-premium long grain rice.*Int. J. Food Sci. Tech*., 35: 257-265.

Chunwongse J., G. B. Martin and S. D. Tanksley.(1993). Pre-germination genotypic screening using PCR amplification of half-seeds. *Theor. Appl. Genet*., 86: 694-698.

Kang H. W., Y. G. Cho, U. H. Yoon and M. Y. Eun (1998).A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed.*Plant Mol. Biol. Rep*. 16: 1-9.

Lucchese C, Dinelli, G, Miggiano A, Lovato, Identification of pepper (*Capsicum spp.*) cultivars by field and electrophoresis tests. (1999).*Seed Sci&Technol*, 27: 37-47.

Pal S., S. Jain and R. K. Jain. (2001). DNA isolation from milled rice samples for PCR based molecular marker analysis. RGN 18: 94.

Rajeshwari, B. C. Viraktamath, N. P. Sarma and R. V. Sonti. (2004). A sequence specific PCR marker for distinguishing rice lines on the basis of wild abortive cytoplasm from their cognate maintainer lines. *Crop Sci*., 44: 920-924.

Ranjitha, H. P., Paramesh, R., Amarnath, M., Shashidhar, H. E. (2016). Influence of Water Regimes on Plant Growth and Seed Yield Attributes of Rice (Oryzasativa L.) under Aerobic and Wetland Conditions. *Environment & Ecology*, 34 (1A): 353—356.

Ranjitha, H. P.; Paramesh, R., Shashidhar, H. E., Nethra, N. and Amruta, N. (2016) Influence of Water Regimes on Reactive Oxygen Species Activity in Rice (Oryzasativa L.)Under Aerobic and Wetland Condition. *Inter.J of Scienti. Progress & Res*, 25 **(3):** 143-149.

Sharma A. D., P. K. Gill and P. Singh. (2002). DNA isolation from dry and fresh samples of polysaccharide-rich plants.*Plant Mol. Biol. Rep*., 20: 415a-415f.

Yashitola J., T. Thirumurugan, R. M. Sundaram, M. K. Naseerullah, M. S. Ramesha, N. P. Sarma and R. V. Sonti. (2002). Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Sci*., 42: 1369-1373.

Zheng K., N. Huang, J. Bennett and G. S. Khush (1995). PCR-based marker assisted selection in rice breeding. IRRI Discussion Paper Series No. 12. International Rice Research Institute, Manila, the Philippines.

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